

## Innovative Foldamers: Engineering Heterochiral Peptides

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**Innovative Foldamers:** The field of foldamer design promises new routes to important compounds for use in sensors, smart materials, and catalysts. The term “foldamer” refers to a molecule that folds into a structurally stable state in solution.<sup>1</sup> Proteins and peptides are an important class of natural foldamers that carry out a host of essential functions in biology, including molecular recognition, information storage, catalysis, and controlled crystallization of inorganic materials. The desire to mimic such functions with synthetic molecules inspires the field of foldamer design.

Of the foldamers under development,  $\beta$  helices — peptide helices containing amino acids with alternating chirality — represent an intriguing and relatively unexplored subclass of peptide-based foldamers. Very few  $\beta$ -helical peptides exist in nature, and all of these compounds adopt their active  $\beta$ -helical structures in hydrophobic membrane environments. However, for many potential biomimetic or bioinspired applications, water or other polar solvents will likely be the medium of choice. In our research, therefore, we pose the question: Can engineered  $\beta$  helices discretely fold in polar media such as methanol, and ultimately water?

**Peptide Engineering:** In designing  $\beta$  helices, we must overcome several challenges, including the tendency of  $\beta$  helices to adopt multiple structures in solution (structural polymorphism), and the tendency of water to disrupt the hydrogen bonds that stabilize folded peptide structures. Structural polymorphism and aggregation are undesirable for functional foldamers as these physical phenomena effectively decrease the concentration of the desired structure, leading to lower activity. To limit structural polymorphism, we developed a method to trap a singular  $\beta$ -helical structure by linking the two strands and forming a cyclic peptide (Fig. 4).<sup>2</sup> The cyclic construction also helps to stabilize the folded structure against the disrupting effect of water. To further stabilize the peptide in polar media, we designed hydrophilic sites and two stabilizing electrostatic interactions into the structure (peptide 1, Fig. 5(a)). After synthesis and purification, we found that the resulting peptide folded stably in the highly polar solvent methanol — an unprecedented achievement in the field of  $\beta$ -helical peptide foldamers.<sup>3</sup>

**Structural Characterization:** Circular dichroism (CD) uses differential adsorption of polarized light

to reveal general structural characteristics in biological molecules. Our initial studies began in methanol, an alcohol solvent often employed as a surrogate for water. In methanol, the CD spectrum is characteristic of a  $\beta$ -helical structure. As we added increasing amounts of water, the peptide partially unfolded into a bracelet-like structure.

We further probed the peptide’s aggregation state and thermal stability by CD. The CD spectra of peptide 1 in methanol and water are independent of concentration, suggesting the peptide is monomeric under the conditions studied. The CD signals in methanol and water do not change appreciably from 5 to 65 °C, indicating both structures are surprisingly thermostable; for comparison, most proteins unfold at 45 °C.

Another technique that allows several different means for establishing molecular structure is nuclear magnetic resonance (NMR). NMR perturbs and monitors the nuclear spins of atoms, which are sensitive to local chemical environments, thus making NMR a powerful technique for investigating the three-dimensional structure of molecules. Computer modeling using NMR data with simulated annealing molecular dynamics (SAMD) generates structures consistent with the experimental measurements — effectively allowing us to determine the coordinates of the atoms in the molecule. Using NMR and SAMD, we were able to generate images of the molecular structures of peptide 1 in methanol (Fig. 5(b)) and water (Fig. 5(c)). In water, the molecule consists of three subgroups all having topography with antiparallel strands and two turn regions; in methanol, the peptide forms the intended structure — a well-defined  $\beta$  helix (Fig. 5(b)).

**Second-Generation Peptide:** Having achieved a  $\beta$ -helical structure in methanol, we next sought to design a new peptide that would adopt a similar structure in water. For this second-generation peptide, we increased the length of the peptide, which we expected would increase the number of helical hydrogen bonds, thus increasing the overall stability of the helical fold. Furthermore, we designed peptide 2 (Fig. 6) to be stabilized by six electrostatic interactions in the expected helical structure. The CD spectrum of peptide 2 in buffered water confirmed the formation of a  $\beta$  helix and validated our approach to the problem. This demonstration of a  $\beta$  helix in water furthers our goal of developing  $\beta$  helices as a versatile class of foldamers.

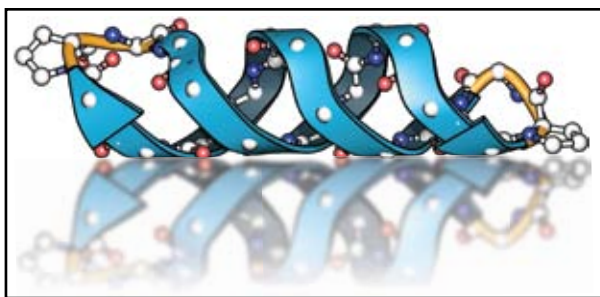
**Conclusions and Outlook:** We have presented the design and biophysical characterization of the first  $\beta$ -helical peptide foldamers that adopt stable structures in polar solvents. By joining the strands with turns, we trapped the peptides into a discrete structural state — a prerequisite for a fully functional foldamer. Our studies prove the  $\beta$  helices can fold stably in polar solvents;

the fundamental principles established in this work will enable the future design of predictable  $\beta$ -helical structures. These innovative heterochiral peptides promise numerous potential applications as sensors, smart materials, and catalysts, thus enhancing NRL's multidisciplinary technology platform.

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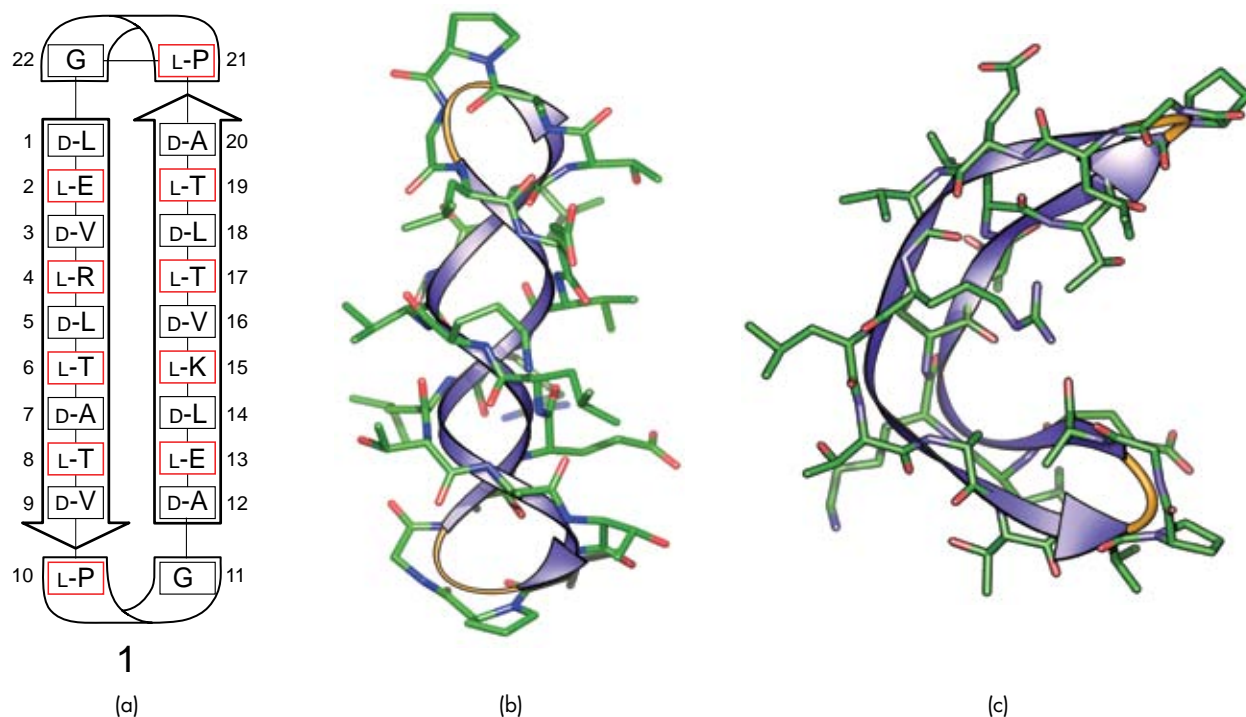
#### References

- <sup>1</sup> S. Hecht and I. Huc, eds., *Foldamers: Structure, Properties, and Applications* (Wiley-VCH, 2007).
- <sup>2</sup> M. Sastry, C. Brown, G. Wagner, and T.D. Clark, "Cyclic Peptide Helices: A Hybrid  $\beta$ -Hairpin,  $\beta$ -Helical Supersecondary Structure," *J. Am. Chem. Soc.* **128**, 10650–10651 (2006).
- <sup>3</sup> J.L. Kulp III and T.D. Clark, "Engineering a  $\beta$ -helical D,L Peptide for Folding in Polar Media," *Chem. Eur. J.*, **2009**, 15(44), 11867–11877.

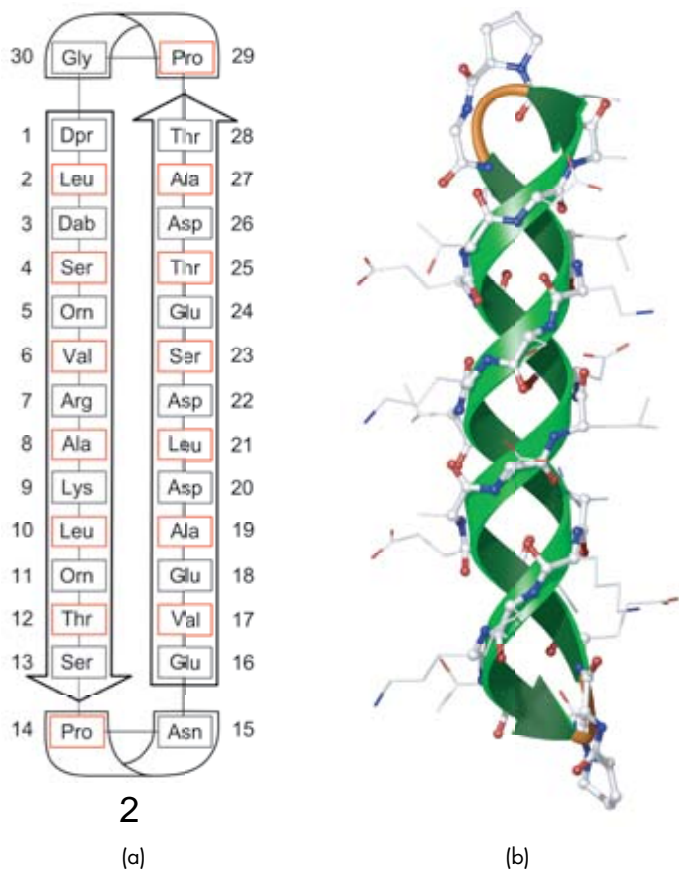


**FIGURE 4**

Peptide backbone structure of a  $\beta$ -helical supersecondary structure developed, synthesized, and characterized at NRL.

**FIGURE 5**

(a) Sequence and numbering scheme for peptide 1 using one-letter codes for amino acid residues; D,L-convention for denoting chirality describes amino acids that are non-superimposable on its mirror image — human hands being the most common example of chirality; D-amino acids and glycine are boxed in black, L-amino acids are boxed in red. (b) and (c) Nuclear magnetic resonance derived structures, computed by simulated annealing molecular dynamics, of peptide 1 in methanol (b) and buffered water (c).

**FIGURE 6**

(a) Sequence and numbering scheme for second-generation peptide 2 using three-letter codes for amino acid residues; D-amino acids and glycine are boxed in black, L-amino acids are boxed in red. (b) Energy minimized model based on CD data.